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Disinfection of endoscopes from *Helicobacter pylori*–positive subjects: Evaluation of the effectiveness of the Chinese Calijing disinfection kit

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Background: The aim of this study was to evaluate the effectiveness of the Calijing disinfection kit (an endoscope disinfection method used in Chinese hospitals) in eradicating *Helicobacter pylori* and assess whether use of the kit in 1994 during endoscopies in the Shandong Intervention Trial (SIT), Shandong, China, could have resulted in iatrogenic transmission of *H pylori*.
Methods: Bacterial culture studies at the Veterans Affairs Medical Center, Houston, Texas, using endoscopes and forceps from 49 *H pylori*-positive patients were performed on contaminated endoscopes before and after disinfection with the Calijing kit.
Results: At least 1 endoscope culture site was *H pylori* positive in 39 of 49 (79.6%) specimens predisinfection, whereas *H pylori* was not isolated from any endoscopic culture site postdisinfection. Non-*H pylori* bacteria and fungi were recovered from 22.6% of the postdisinfection cultures.
Conclusion: Although no viable *H pylori* were recovered following the disinfection procedures, levels of *H pylori* below the detection threshold of the bacteriologic assay may have contributed to an increase in *H pylori* seroprevalence noted in the SIT. In addition, the kit was unable to provide disinfection against non-*H pylori* organisms, suggesting the need to adhere to internationally accepted disinfection procedures for endoscope reprocessing. (Am J Infect Control 2005;33:197-201.)

Helicobacter pylori (*H pylori*) is one of the most common bacterial infections in humans worldwide and has been recognized as a major cause of gastritis.[1] It is also considered a risk factor for duodenal ulcer disease, gastric ulcer disease, and gastric lymphoma and has been linked to gastric cancer.[2-4] Most epidemiologic data support a person-to-person mode of transmission, but nosocomial transmission of *H pylori* is the only proven mode of transmission.

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We have been following the perplexing finding that an unusually high percentage of persons originally classified as *H pylori* seronegative in 1994 in the Shandong Intervention Trial (SIT), a blinded, randomized, 2^3 factorial trial of 3411 subjects in 13 rural villages in Linqu County, Shandong Province, China, were found to have either a positive urease breath test (UBT) (39.7%) or a positive serologic test (41.3%) in 1996.[5] This change in serostatus corresponds to an annual seroconversion rate of 23.4% between 1994 and 1996 compared with an annual seroconversion rate of 4.2% between 1989 and 1994. The annual rate of seroconversion in adult populations in developed countries appears to be small (on the order of 0.2%-1.0%); whereas higher rates (on the order of 6.4%-7.3%) have been documented among adults in less developed countries.[6-8]

One possibility for the high rate of seroconversion is that *H pylori* baseline serostatus could have been misclassified because *H pylori* was endemic in our study population (approximately 67% of the study population was seropositive at baseline) and that some of the ''seroconvertors'' may actually have habored the *H pylori* organism in 1994 and, therefore, were not really ''negative'' as indicated by their baseline serology.

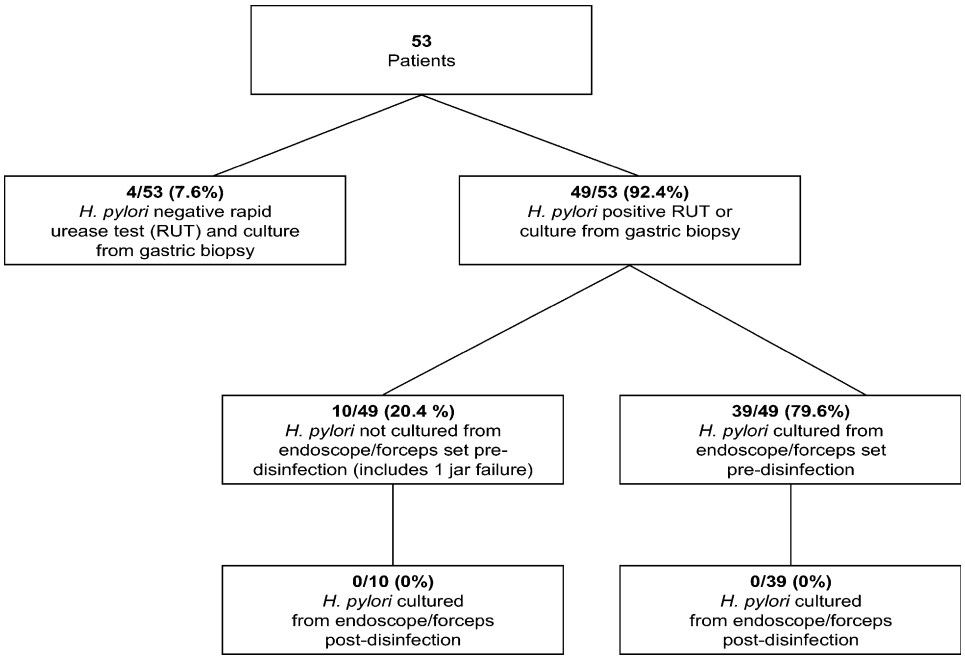

Fig 1. *Helicobacter pylori* contamination pre- and postdisinfection with the Calijing disinfection kit.

Another possibility is that some people who underwent endoscopy in 1994 as part of a previous cross-sectional study of gastric lesion progression (GLP) conducted from 1989 to 1994 might have been infected as a result of that endoscopy. Up to 75 endoscopies were performed each day, using 3 endoscopists and 6 endoscopes. The endoscope disinfection procedures used in Shandong in 1989 for the GLP study met the standards of the Working Party Report to the World Congresses of Gastroenterology, Sidney, 1990.[9,10] However, the endoscope disinfection procedures used in 1994 were modified by Chinese collaborators at the Beijing Institute for Cancer Research (BICR). A 1:2000 chlorhexidine solution, a skin antiseptic not approved for medical device reprocessing in the United States, was utilized for rinsing the endoscope. Instead of soaking the endoscopes in Cidex (Johnson & Johnson, Advanced Sterilization Products, Irvine, CA) (2.4% alkaline glutaraldehyde in water) for 10 minutes, the endoscopes were cleaned using Calijing disinfection kits (synthetic sponges saturated with 2.4% glutaraldehyde and used to wipe the outside of the endoscope) manufactured in a factory in Tianjin, China. Forceps were cleaned and disinfected by soaking for 10 minutes in Cidex (Johnson & Johnson) squeezed from the Calijing sponge. Although the Calijing kit is currently used in approximately 1500 hospitals in China for endoscope disinfection, there have not yet been any reports on its efficacy against transmission of *H pylori* from contaminated endoscopes. The purpose of this study is to assess whether use of the Calijing disinfection kit could have resulted in iatrogenic transmission of *H pylori* during the 1994 endoscopies and to present data on the effectiveness of the Calijing disinfection kit.

MATERIALS AND METHODS

Fifty-three patients at the Veterans Affairs Medical Center, Houston, Texas, underwent medically indicated gastric endoscopy and had rapid urease testing (RUT) (Hp*fast*) and culture in gastric biopsies. The endoscopes and forceps that had been used in these patients were evaluated for *H pylori* and other bacteria and fungi before and after disinfection. The endoscopy suite was adjacent to the microbiologic laboratory, allowing immediate microbiologic evaluation of the endoscopes. Human subjects and institutional review were not required for this laboratory study because data were not linked to the patient's name or hospital record.

Following endoscopy, but before disinfection, the following procedure was performed on each endoscope. One entire side of the endoscope was swabbed with a sterile cotton swab (moistened in transport medium) and plated onto a nonselective plate of Mueller-Hinton agar medium containing 7% horse blood and a selective plate of Mueller-Hinton agar culture medium containing 7% horse blood and *H pylori* selective antibiotics to prevent overgrowth of other bacteria. The biopsy channel was flushed with a small amount of saline, and the washings were plated onto a nonselective and a selective agar plate. Next, the inner and

outer surfaces of 1 jaw of the forceps were swabbed with a moistened sterile cotton swab and plated onto a nonselective and a selective agar plate.

The endoscope and forceps were then disinfected utilizing the same techniques as those employed by the BICR in 1994, as described below. Using protective equipment to protect hands and eyes, the endoscope and forceps were flushed and rinsed with water. The biopsy and water channels were flushed with water to clear the gastric mucus, using 3 changes of sterile water consisting of 10 forceful, 30 mL volumes each. The channels and forceps were brushed, and the rim of the suction ports was swabbed clean with sterile cotton swabs. The steps above were repeated using fresh chlorhexidine 1:2000 solution in water. First, the outside of the endoscope and the forceps were rinsed with chlorhexidine 1:2000 solution in water. Next, the endoscope channels were rinsed with 3 changes of 10 forceful, 30 mL volumes each of chlorhexidine solution. The Calijing disinfection kit, consisting of 1 dry sponge and 2 Cidex-treated sponges sealed in foil, was opened under the fume hood. The dry sponge was used to wipe the outside of the endoscope. The Cidex-treated sponges were then opened. The forceps were soaked in disinfection solution squeezed from the Cidex-treated sponges for 10 minutes. During the soaking of the forceps, the endoscope was disinfected by placing it between the 2 sponges and squeezing the sponges to release the Cidex solution. The outside of the endoscope was wiped for 5 minutes using the Cidex-treated sponges. Approximately 25 mL Cidex solution expressed from the sponges was sucked into the biopsy channel and flushed repeatedly until all the solution was used.

Following disinfection, the endoscope, biopsy channel (3 changes of 10 forceful, 30 mL volumes each), and forceps were rinsed with sterile water several times. The endoscope and forceps were dried with sterile gauze and processed for culture. The outer wall and head of the entire used endoscope were swabbed with a moistened sterile cotton swab and plated onto a nonselective and a selective agar plate. The inside of the biopsy channel was wiped with a brush, and the brush was plated directly onto a nonselective and a selective agar plate. The inner and outer surfaces of both jaws of the forceps were swabbed with a moistened sterile cotton swab and plated onto a nonselective and a selective agar plate. All 12 agar plates were labeled with the subject's ID number, the date the specimen was collected, the source of the specimen (surface of endoscope, endoscope biopsy channel, or forceps), and the disinfection status (before or after disinfection).

Positive control plates were prepared by performing quantitative cultures on known concentrations of *H pylori* (spectrophotometric analysis, at 625 nm equivalent to an optical density of 0.18-0.20). Assuming that each milliliter of culture broth contained 3×10^8 bacteria, 8 serial 1:10 dilutions were prepared in sterile saline, and 100 μL culture broth from each dilution were plated onto non-selective Mueller-Hinton agar plates containing 7% horse blood. New positive control plates were prepared for each batch of subject plates sent for culture.

All sample plates were placed in an anaerobic container that contained a CampyPak Plus gas-generating envelope (Becton Dickenson BBL, Cockeysville, MD) and incubated in a 37°C incubator for 10 days. Concurrent positive control plates were included to determine whether assay conditions allowed for growth. The extent of growth of microorganisms was first observed at day 3 of incubation then daily for the remaining 7 days of incubation. If no colony was visible by day 10, the culture was considered *H pylori* negative. Microorganisms resembling *H pylori* based on the typical colony morphology (translucent, convex, circular, entire edge, possible small zone of B-hemolysis around colonies), Gram's stain reaction (negative), cell morphology (slender curved to spiral rods, some coccoid forms and horse-shoe shapes) were further tested for urease, catalase, and oxidase positivity to confirm identity. Laboratory personnel were blinded to the extent possible to the source of specimens and to their disinfection status (total blinding was impossible given the level of labeling required by the protocol).

A biopsy culture slide study was conducted to evaluate the possibility that *H pylori* serostatus could have been misclassified at baseline in 1994. A random sample of 48 subjects with negative baseline *H pylori* serology in 1994 and positive serology in 1996 (group A), 39 with positive serology at baseline in 1994 (group B), and 42 with negative serology at baseline in 1994 and in 1996 (group C) was selected. Four biopsy tissue blocks (2 from the antrum and 2 from the corpus) were retrieved from each subject, and a slide was made from each block at the BICR. Each slide was treated with Diff Quik, a standard stain for identification of *H pylori*.[11] The slide was then reviewed by one of us (H.E.) under the microscope for the presence of *H pylori*. If *H pylori* was found on any of the 4 slides, the subjects was considered *H pylori* positive at baseline.

RESULTS

Review of data from the slide study revealed that 10.5% of group A and 9.5% of group C had identifiable *H pylori* on 1994 slides, despite the fact that these groups were seronegative, compared with 80% of group B. These results indicate that the false-negative rate at

baseline was about 10% and insufficient to explain the high rate of seroconversions between 1994 and 1996.

The results of the endoscope study are presented in Fig 1. A total of 53 patients were enrolled in the study. Of these, 49 of 53 (92.5%) subjects had *H pylori* demonstrated either from gastric biopsies by culture or by the RUT. The Hp*fast* rapid urease test was positive in 48 of these subjects, whereas *H pylori* biopsy cultures were positive in all 49 subjects. Included in this Figure is 1 instance in which no organisms were recovered from any site, both predisinfection and postdisinfection, and in which positive controls failed to grow, suggesting that a ''jar failure'' may have occurred during the incubation period for this subject. At least 1 endoscope or forceps culture site was *H pylori* positive in 39 of 49 (79.6%) of these RUT-positive specimens. Individual sites varied in their recovery rate of *H pylori*, with the channel cultures having the greatest recovery rate (61.2%, 30/49) compared with the culture of the outside endoscope (55.1%, 27/49) or the forceps (6.1%, 3/49). At least 1 culture site was positive for non-*H pylori* bacteria or fungi before disinfection in 52 of 53 (98.1%) of the endoscopes. The outside of the endoscope yielded the highest level of contaminating microorganisms (98.1%, 52/53), the channel was positive in 41 of 53 (77.4%), and the forceps were positive for contaminating microbes in 8 of 53 (15.1%).

H pylori were not isolated from any culture site from the 53 endoscope sets after disinfection with the Calijing disinfection kit. However, at least 1 culture site was positive for non-*H pylori* organisms in 12 of 53 (22.6%) of the endoscope sets disinfected with the Calijing disinfection kit. Cultures were positive for non-*H pylori* organisms from the endoscope in 8 of 53 (15.1%) subjects, from the biopsy channel in 8 of 53 (15.1%) subjects, and from the forceps in 4 of 53 (7.5%) subjects. Gram-negative rods were the most prevalent organisms recovered after disinfection from the 3 culture sites. Fungal contamination was also present.

H pylori were not cultured after disinfection from any of the 39 endoscope/forcep sets from which *H pylori* had been isolated before disinfection (Fig 1). Based on these data, the upper 95% confidence limit on the probability of finding *H pylori* after disinfection on instruments in which *H pylori* were cultured before disinfection was 0.074. Likewise, the upper confidence limit of the probability of detecting *H pylori* in culture after disinfection among 49 subjects with *H pylori* demonstrated either from gastric biopsies by culture or by the RUT was 0.059, based on 0 of 49 positive results (Fig 1). Even if disinfection allowed 7.4% (the upper 95% confidence limit) of *H pylori*-positive scopes to remain positive, this could not account for the approximately 40% increase in the *H pylori* seropositivity noted between 1994 and 1996 in the SIT. The fact that only approximately 67% of the Chinese population was seropositive in 1994 suggests that, at most, $0.67 \times 0.074 = 5\%$ of the seroconversions could be due to imperfect disinfection because, on average, only approximately 67% of the instruments would have been infected by the previous user.

DISCUSSION

Because of the complex structure of the endoscope and the difficulty in disinfecting it, there is a possibility of iatrogenic infection in patients following endoscopy. In fact, nosocomial transmission of *H pylori* is the only proven mode of transmission.[4] The rate of iatrogenic infection may reach 1% or higher in areas of the world utilizing improper disinfection techniques.[12-14] Proper cleaning requires use of a detergent and brushing (and often use of an enzymatic cleaner) to remove blood, mucous, and tissue from the endoscope channels prior to disinfection with glutaraldehyde; sterilization of the forceps or preferably use of disposable forceps is essential.[10,12,15] Studies in our laboratory in Texas by two of us (M.O. and H.E.) demonstrate that a dose of less than 10^4 viable bacteria is required to achieve infection in *H pylori* naïve individuals.[16]

In the current study, the overall predisinfection recovery rate of *H pylori* from endoscopes/forceps used to obtain biopsy cultures from known *H pylori*-positive patients was 79.6% (39/49), considerably higher than the 42.2% (54/128) predisinfection recovery rate in a recent study by Nürnberg et al.[17] By comparison, none of the postdisinfection samples in our study was positive for *H pylori*. In the study by Nürnberg et al that utilized routine manual cleaning and immersion in 2% gluteraldehyde for 15 minutes, the postdisinfection *H pylori* recovery rate was 1.9% (1/54), a number consistent with our calculated upper confidence limit of 7.4%.[17] In contrast, the recovery rate of *H pylori* using the standard culture procedures for gastric mucosal biopsies was 92.5% in our study.

Desiccation of mucous at the time of processing, because of the need to await confirmatory data that the patient was *H pylori* positive by RUT, may account for failure to culture *H pylori* before disinfection from the endoscopes or forceps in 10 of the 49 subjects with *H pylori* demonstrated in biopsies. In every case, the forceps jaws were dry when cultured, which may account for the low *H pylori* recovery rate from forceps. ''Washing'' the forceps in transport medium to remove adherent gastric biopsy tissue may have dislodged *H pylori*.

All of the predisinfection cultures, except one with probable jar failure, yielded non-*H pylori* contaminants. These organisms ranged from gram-negative bacilli to filamentous fungi. Non-*H pylori* organisms on

the endoscopic cultures but not present on cultures of the gastric biopsies were regarded as inadvertent contaminants and were not counted. The ability of these non-*H pylori* microbes to survive for extended periods outside the stomach contrasts with the less adaptable *H pylori*.

CONCLUSION

The Calijing kit effectively disinfected the endoscope and forceps of *H pylori*; however, it failed to eliminate non-*H pylori* organisms, which were recovered from 22.6% of the specimens after disinfection. The inability to achieve disinfection against all organisms raises the possibility that a small number of *H pylori* below the detectability of our culture methods may have survived disinfection. Therefore, our data do not completely rule out the possibility of iatrogenic transmission of *H pylori* following disinfection with the Calijing kit and suggest the need to adhere to accepted guidelines for endoscope reprocessing.[18,19]

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